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(71) Applicant: **ARIUS RESEARCH, INC.** [CA/CA]; 6354 Viscount Road, Mississauga, Ontario L4V 1H3 (CA).

(72) Inventors: **YOUNG, David, S., F.**; 51 Baldwin Street, Apt. 3, Toronto, Ontario M5T 1L1 (CA). **TAKAHASHI, Miyoko**; 65 Franklin Avenue, Toronto, Ontario M2M 1B8 (CA).

(74) Agent: **SINGLEHURST, John, C.**; Finlayson & Singlehurst, 70 Gloucester Street, Ottawa, Ontario K2P 0A2 (CA).

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(54) Title: INDIVIDUALIZED ANTI-CANCER ANTIBODIES

(57) Abstract: The present invention relates to a method for producing patient specific anti-cancer antibodies using a novel paradigm of screening. By segregating the anti-cancer antibodies using cancer cell cytotoxicity as end point, the process makes possible the production of anti-cancer antibodies customized for the individual patient that can be used for therapeutic and diagnostic purposes.

INDIVIDUALIZED ANTI-CANCER ANTIBODIES

Reference to Related Applications:

This application is a continuation-in-part of application S.N. 09/415,278, filed October 8, 1999, now U.S. Patent 6,180,357, the contents of which are herein incorporated by reference.

Field of the Invention:

This invention relates to the production of anti-cancer antibodies customized for the individual patient which may be combined with chemotherapeutic agents that can be used for therapeutic and diagnostic purposes. The invention further relates to the process by which the antibodies are made and to their methods of use.

Background of the Invention:

Each individual who presents with cancer is unique and has a cancer that is as different from other cancers as that person's identity. Despite this, current therapy treats all patients with the same type of cancer, at the same stage, in the same way. At least 30% of these patients will fail the first line therapy, thus leading to further rounds of treatment and the increased probability of treatment failure, metastases, and ultimately, death. A superior approach to treatment would be the customization of therapy for the particular individual. The only current therapy which lends itself to customization is surgery. Chemotherapy and radiation treatment can not be tailored to the patient, and surgery by itself, in most cases is inadequate for producing cures.

With the advent of monoclonal antibodies, the possibility of developing methods for customized therapy became more realistic since each antibody can be directed to a single epitope. Furthermore, it is possible to produce a combination of antibodies that are directed to

the constellation of epitopes that uniquely define a particular individual's tumor.

Having recognized that a significant difference between cancerous and normal cells is that cancerous cells contain antigens that are specific to transformed cells, the scientific community has long held that monoclonal antibodies can be designed to specifically target transformed cells by binding specifically to these cancer antigens; thus giving rise to the belief that monoclonal antibodies can serve as "Magic Bullets" to eliminate cancer cells.

At the present time, however, the cancer patient usually has few options of treatment. The regimented approach to cancer therapy has produced improvements in global survival and morbidity rates. However, to the particular individual, these improved statistics do not necessarily correlate with an improvement in their personal situation.

Thus, if a methodology was put forth which enabled the practitioner to treat each tumor independently of other patients in the same cohort, this would permit the unique approach of tailoring therapy to just that one person. Such a course of therapy would, ideally, increase the rate of cures, and produce better outcomes, thereby satisfying a long-felt need.

Historically, the use of polyclonal antibodies has been used with limited success in the treatment of human cancers. Lymphomas and leukemias have been treated with human plasma, but there were few prolonged remission or responses. Furthermore, there was a lack of reproducibility and there was no additional benefit compared to chemotherapy. Solid tumors such as breast cancers, melanomas and renal cell carcinomas have also been treated with human blood, chimpanzee serum, human

plasma and horse serum with correspondingly unpredictable and ineffective results.

There have been many clinical trials of monoclonal antibodies for solid tumors. In the 1980s there were at least four clinical trials for human breast cancer which produced only one responder from at least 47 patients using antibodies against specific antigens or based on tissue selectivity. It was not until 1998 that there was a successful clinical trial using a humanized anti-her 2 antibody in combination with Cisplatin. In this trial 37 patients were accessed for responses of which about a quarter had a partial response rate and another half had minor or stable disease progression.

The clinical trials investigating colorectal cancer involve antibodies against both glycoprotein and glycolipid targets. Antibodies such as 17-1A, which has some specificity for adenocarcinomas, had undergone Phase 2 clinical trials in over 60 patients with only one patient having a partial response. In other trials, use of 17-1A produced only one complete response and two minor responses among 52 patients in protocols using additional cyclophosphamide. Other trials involving 17-1A yielded results that were similar. The use of a humanized murine monoclonal antibody initially approved for imaging also did not produce tumor regression. To date there has not been an antibody that has been effective for colorectal cancer. Likewise there have been equally poor results for lung cancer, brain cancers, ovarian cancers, pancreatic cancer, prostate cancer, and stomach cancer. There has been some limited success in the use of anti-GD3 monoclonal antibody for melanoma. Thus, it can be seen that despite successful small animal studies that are a prerequisite for human clinical trials, the antibodies that have been tested have been for the most part ineffective.

Prior Patents:

U.S. Patent No. 5,750,102 discloses a process wherein cells from a patient's tumor are transfected with MHC genes which may be cloned from cells or tissue from the patient. These transfected cells are then used to vaccinate the patient.

U.S. Patent No. 4,861,581 discloses a process comprising the steps of obtaining monoclonal antibodies that are specific to an internal cellular component of neoplastic and normal cells of the mammal but not to external components, labeling the monoclonal antibody, contacting the labeled antibody with tissue of a mammal that has received therapy to kill neoplastic cells, and determining the effectiveness of therapy by measuring the binding of the labeled antibody to the internal cellular component of the degenerating neoplastic cells. In preparing antibodies directed to human intracellular antigens, the patentee recognizes that malignant cells represent a convenient source of such antigens.

U.S. Patent No. 5,171,665 provides a novel antibody and method for its production. Specifically, the patent teaches formation of a monoclonal antibody which has the property of binding strongly to a protein antigen associated with human tumors, e.g. those of the colon and lung, while binding to normal cells to a much lesser degree.

U.S. Patent No. 5,484,596 provides a method of cancer therapy comprising surgically removing tumor tissue from a human cancer patient, treating the tumor tissue to obtain tumor cells, irradiating the tumor cells to be viable but non-tumorigenic, and using these cells to prepare a vaccine for the patient capable of inhibiting recurrence of the primary tumor while simultaneously inhibiting metastases. The patent teaches the development of monoclonal antibodies which are reactive with surface antigens of tumor cells. As set forth at col. 4, lines 45

et seq., the patentees utilize autochthonous tumor cells in the development of monoclonal antibodies expressing active specific immunotherapy in human neoplasia.

U.S. Patent No. 5,693,763 teaches a glycoprotein antigen characteristic of human carcinomas and not dependent upon the epithelial tissue of origin.

U.S. Patent No. 5,783,186 is drawn to Anti-Her2 antibodies which induce apoptosis in Her2 expressing cells, hybridoma cell lines producing the antibodies, methods of treating cancer using the antibodies and pharmaceutical compositions including said antibodies.

U.S. Patent No. 5,849,876 describes new hybridoma cell lines for the production of monoclonal antibodies to mucin antigens purified from tumor and non-tumor tissue sources.

U.S. Patent No. 5,869,268 is drawn to a method for producing a human lymphocyte producing an antibody specific to a desired antigen, a method for producing a monoclonal antibody, as well as monoclonal antibodies produced by the method. The patent is particularly drawn to the production of an anti-HD human monoclonal antibody useful for the diagnosis and treatment of cancers.

U.S. Patent No. 5,869,045 relates to antibodies, antibody fragments, antibody conjugates and single chain immunotoxins reactive with human carcinoma cells. The mechanism by which these antibodies function is two-fold, in that the molecules are reactive with cell membrane antigens present on the surface of human carcinomas, and further in that the antibodies have the ability to internalize within the carcinoma cells, subsequent to binding, making them especially useful for forming antibody-drug and antibody-toxin conjugates. In their unmodified form the antibodies also manifest cytotoxic properties at specific concentrations.

U.S. Patent No. 5,780,033 discloses the use of autoantibodies for tumor therapy and prophylaxis. However,

this antibody is an antinuclear autoantibody from an aged mammal. In this case, the autoantibody is said to be one type of natural antibody found in the immune system. Because the autoantibody comes from "an aged mammal", there is no requirement that the autoantibody actually comes from the patient being treated. In addition the patent discloses natural and monoclonal antinuclear autoantibody from an aged mammal, and a hybridoma cell line producing a monoclonal antinuclear autoantibody.

Summary of the Invention:

This application teaches a method for producing patient specific anti-cancer antibodies using a novel paradigm of screening. These antibodies can be made specifically for one tumor and thus make possible the customization of cancer therapy. Within the context of this application, anti-cancer antibodies having either cell-killing (cytotoxic) or cell-growth inhibiting (cytostatic) properties will hereafter be referred to as cytotoxic. These antibodies can be used in aid of staging and diagnosis of a cancer, and can be used to treat tumor metastases.

The prospect of individualized anti-cancer treatment will bring about a change in the way a patient is managed. A likely clinical scenario is that a tumor sample is obtained at the time of presentation, and banked. From this sample, the tumor can be typed from a panel of pre-existing anti-cancer antibodies. The patient will be conventionally staged but the available antibodies can be of use in further staging the patient. The patient can be treated immediately with the existing antibodies, and a panel of antibodies specific to the tumor can be produced either using the methods outlined herein or through the use of phage display libraries in conjunction with the screening methods herein disclosed. All the antibodies generated will be added to the library of anti-cancer

antibodies since there is a possibility that other tumors can bear some of the same epitopes as the one that is being treated.

In addition to anti-cancer antibodies, the patient can elect to receive the currently recommended therapies as part of a multi-modal regimen of treatment. The fact that the antibodies isolated via the present methodology are relatively non-toxic to non-cancerous cells allow combinations of antibodies at high doses to be used, either alone, or in conjunction with conventional therapy. The high therapeutic index will also permit re-treatment on a short time scale that should decrease the likelihood of emergence of treatment resistant cells.

If the patient is refractory to the initial course of therapy or metastases develop, the process of generating specific antibodies to the tumor can be repeated for re-treatment. Furthermore, the anti-cancer antibodies can be conjugated to red blood cells obtained from that patient and re-infused for treatment of metastases. There have been few effective treatments for metastatic cancer and metastases usually portend a poor outcome resulting in death. However, metastatic cancers are usually well vascularized and the delivery of anti-cancer antibodies by red blood cells can have the effect of concentrating the antibodies at the site of the tumor. Even prior to metastases, most cancer cells are dependent on the host's blood supply for their survival and anti-cancer antibody conjugated red blood cells can be effective against *in situ* tumors, too. Alternatively, the antibodies may be conjugated to other hematogenous cells, e.g. lymphocytes, macrophages, monocytes, natural killer cells, etc.

There are five classes of antibodies and each is associated with a function that is conferred by its heavy chain. It is generally thought that cancer cell killing

by naked antibodies are mediated either through antibody dependent cellular cytotoxicity or complement dependent cytotoxicity. For example murine IgM and IgG2a antibodies can activate human complement by binding the C-1 component of the complement system thereby activating the classical pathway of complement activation which can lead to tumor lysis. For human antibodies the most effective complement activating antibodies are generally IgM and IgG1. Murine antibodies of the IgG2a and IgG3 isotype are effective at recruiting cytotoxic cells that have Fc receptors which will lead to cell killing by monocytes, macrophages, granulocytes and certain lymphocytes. Human antibodies of both the IgG1 and IgG3 isotype mediate ADCC.

Another possible mechanism of antibody mediated cancer killing may be through the use of antibodies that function to catalyze the hydrolysis of various chemical bonds in the cell membrane and its associated glycoproteins or glycolipids, so-called catalytic antibodies.

There are two additional mechanisms of antibody mediated cancer cell killing which are more widely accepted. The first is the use of antibodies as a vaccine to induce the body to produce an immune response against the putative cancer antigen that resides on the tumor cell. The second is the use of antibodies to target growth receptors and interfere with their function or to down regulate that receptor so that effectively its function is lost.

Accordingly, it is an objective of the invention to teach a method for producing anti-cancer antibodies from cells derived from a particular individual which are cytotoxic with respect to cancer cells while simultaneously being relatively non-toxic to non-cancerous cells.

It is an additional objective of the invention to produce novel anti-cancer antibodies.

It is a further objective of the instant invention to produce anti-cancer antibodies whose cytotoxicity is mediated through antibody dependent cellular toxicity.

It is yet an additional objective of the instant invention to produce anti-cancer antibodies whose cytotoxicity is mediated through complement dependent cellular toxicity.

It is still a further objective of the instant invention to produce anti-cancer antibodies whose cytotoxicity is a function of their ability to catalyze hydrolysis of cellular chemical bonds.

Still an additional objective of the instant invention is to produce anti-cancer antibodies useful as a vaccine to produce an immune response against putative cancer antigen residing on tumor cells.

A further objective of the instant invention is the use of antibodies to target cell membrane proteins, such as growth receptors, cell membrane pumps and cell anchoring proteins, thereby interfering with or down regulating their function.

Yet an additional objective of the instant invention is the production of anti-cancer antibodies whose cell-killing utility is concomitant with their ability to effect a conformational change in cellular proteins such that a signal will be transduced to initiate cell-killing.

A still further objective of the instant invention is to produce anti-cancer antibodies which are useful for diagnosis, prognosis, and monitoring of cancer, e.g. production of a panel of therapeutic anti-cancer antibodies to test patient samples to determine if they contain any suitable antibodies for therapeutic use.

Yet another objective of the instant invention is to produce novel antigens, associated with cancer processes, which can be discovered by using anti-cancer antibodies derived by the process of the instant invention. These

antigens are not limited to proteins, as is generally the case with genomic data; they may also be derived from carbohydrates or lipids or combinations thereof.

Other objects and advantages of this invention will become apparent from the following description wherein are set forth, by way of illustration and example, certain embodiments of this invention.

Detailed Description of the Invention:

It is to be understood that while a certain form of the invention is illustrated, it is not to be limited to the specific form or arrangement herein described and shown. It will be apparent to those skilled in the art that various changes may be made without departing from the scope of the invention and the invention is not to be considered limited to what is shown and described in the specification.

One of the potential benefits of monoclonal antibodies with respect to the treatment of cancer is their ability to specifically recognize single antigens. It was thought that in some instances cancer cells possess antigens that were specific to that kind of transformed cell. It is now more frequently believed that cancer cells have few unique antigens, rather, they tend to over-express a normal antigen or express fetal antigens. Nevertheless, the use of monoclonal antibodies provided a method of delivering reproducible doses of antibodies to the patient with the expectation of better response rates than with polyclonal antibodies.

Traditionally, monoclonal antibodies have been made according to fundamental principles laid down by Kohler and Milstein. Mice are immunized with antigens, with or without, adjuvants. The splenocytes are harvested from the spleen for fusion with immortalized hybridoma partners. These are seeded into microtitre plates where

they can secrete antibodies into the supernatant that is used for cell culture. To select from the hybridomas that have been plated for the ones that produce antibodies of interest the hybridoma supernatants are usually tested for antibody binding to antigens in an ELISA (enzyme linked immunosorbent assay) assay. The idea is that the wells that contain the hybridoma of interest will contain antibodies that will bind most avidly to the test antigen, usually the immunizing antigen. These wells are then subcloned in limiting dilution fashion to produce monoclonal hybridomas. The selection for the clones of interest is repeated using an ELISA assay to test for antibody binding. Therefore, the principle that has been propagated is that in the production of monoclonal antibodies the hybridomas that produce the most avidly binding antibodies are the ones that are selected from among all the hybridomas that were initially produced. That is to say, the preferred antibody is the one with highest affinity for the antigen of interest.

There have been many modifications of this procedure such as using whole cells for immunization. In this method, instead of using purified antigens, entire cells are used for immunization. Another modification is the use of cellular ELISA for screening. In this method instead of using purified antigens as the target in the ELISA, fixed cells are used. In addition to ELISA tests, complement mediated cytotoxicity assays have also been used in the screening process. However, antibody-binding assays were used in conjunction with cytotoxicity tests. Thus, despite many modifications, the process of producing monoclonal antibodies relies on antibody binding to the test antigen as an endpoint.

Most antibodies directed against cancer cells have been produced using the traditional methods outlined above. These antibodies have been used both therapeutically and diagnostically. In general, for both

these applications, the antibody has been used as the targeting agent that delivers a payload to the site of the cancer. These antibody conjugates can either be radioactive, toxic, or serve as an intermediary for further delivery of a drug to the body, such as an enzyme or biotin. Furthermore, it was widely held, until recently, that naked antibodies had little effect *in vivo*. Both HERCEPTIN and RITUXIMAB are humanized murine monoclonal antibodies that have recently been approved for human use by the FDA. However, both these antibodies were initially made by assaying for antibody binding and their direct cytotoxicity was not the primary goal during the production of hybridomas. Any tendency for these antibodies to produce tumor cell killing is thus through chance, not by design.

Although the production of monoclonal antibodies have been carried out using whole cell immunization for various applications the screening of these hybridomas have relied on either putative or identified target antigens or on the selectivity of these hybridomas for specific tissues. It is axiomatic that the best antibodies are the ones with the highest binding constants. This concept originated from the basic biochemical principle that enzymes with the highest binding constants were the ones that were the most effective for catalyzing a reaction. This concept is applicable to receptor ligand binding where the drug molecule binding to the receptor with the greatest affinity usually has the highest probability for initiating or inhibiting a signal. However, this may not always be the case since it is possible that in certain situations there may be cases where the initiation or inhibition of a signal may be mediated through non-receptor binding. The information conveyed by a conformational change induced by ligand binding can have many consequences such as a signal transduction, endocytosis, among the others. The ability to produce a

conformational change in a receptor molecule may not necessarily be due to the filling of a ligand receptor pocket but may occur through the binding of another extra cellular domain or due to receptor clustering induced by a multivalent ligand.

The production of antibodies to produce cell killing need not be predicated upon screening of the hybridomas for the best binding antibodies. Rather, although not advocated by those who produce monoclonal antibodies, the screening of the hybridoma supernatants for cell killing or alternatively for cessation of growth of the cancerous cells may be selected as a desirable endpoint for the production of cytotoxic or cytostatic antibodies. It is well understood that the *in-vivo* antibodies mediate their function through the Fc portions and that the utility of the therapeutic antibody is determined by the functionality of the constant region or attached moieties. In this case the FAb portion of the antibody, the antigen-combining portion, will confer to the antibody its specificity and the Fc portion its functionality. The antigen combining site of the antibody can be considered to be the product of a natural combinatorial library. The result of the rearrangement of the variable region of the antibody can be considered a molecular combinatorial library where the output is a peptide. Therefore, the sampling of this combinatorial library can be based on any parameter. Like sampling a natural compound library for antibiotics, it is possible to sample an antibody library for cytotoxic or cytostatic compounds.

The various endpoints in a screen must be differentiated from each other. For example, the difference between antibody binding to the cell is distinct from cell killing. Cell killing (cytotoxicity) is distinct from the mechanisms of cell death such as oncosis or apoptosis. There can be many processes by which cell death is achieved and some of these can lead either to

oncosis or apoptosis. There is speculation that there are other cell death mechanisms other than oncosis or apoptosis but regardless of how the cell arrives at death there are some commonalities of cell death. One of these is the absence of metabolism and another is the denaturation of enzymes. In either case vital stains will fail to stain these cells. These endpoints of cell death have been long understood and predate the current understanding of the mechanisms of cell death. Furthermore, there is the distinction between cytotoxic effects where cells are killed and cytostatic effects where the proliferation of cells are inhibited.

In a preferred embodiment of the present invention, the assay is conducted by focusing on cytotoxic activity toward cancerous cells as an end point. In a preferred embodiment, a live /dead assay kit , for example the LIVE/DEAD® Viability/Cytotoxicity Assay Kit (L-3224) by Molecular Probes, is utilized. The Molecular Probes kit provides a two-color fluorescence cell viability assay that is based on the simultaneous determination of live and dead cells with two probes that measure two recognized parameters of cell viability – intracellular esterase activity and plasma membrane integrity. The assay principles are general and applicable to most eukaryotic cell types, including adherent cells and certain tissues, but not to bacteria or yeast. This fluorescence-based method of assessing cell viability is preferred in place of such assays as trypan blue exclusion, Cr release and similar methods for determining cell viability and cytotoxicity.

In carrying out the assay, live cells are distinguished by the presence of ubiquitous intracellular esterase activity, determined by the enzymatic conversion of the virtually nonfluorescent cell-permeant CALCEIN AM to the intensely fluorescent Calcein. The polyanionic dye Calcein is well retained within live cells, producing an

intense uniform green fluorescence in live cells (ex/em ~495 nm/~515 nm). EthD-1 enters cells with damaged membranes and undergoes a 40-fold enhancement of fluorescence upon binding to nucleic acids, thereby producing a bright red fluorescence in dead cells (ex/em ~495 nm/~635 nm). EthD-1 is excluded by the intact plasma membrane of live cells. The determination of cell viability depends on these physical and biochemical properties of cells. Cytotoxic events that do not affect these cell properties may not be accurately assessed using this method. Background fluorescence levels are inherently low with this assay technique because the dyes are virtually nonfluorescent before interacting with cells.

In addition to the various endpoints for screening, there are two other major characteristics of the screening process. The library of antibody gene products is not a random library but is the product of a biasing procedure. In the examples below, the biasing is produced by immunizing mice with fixed cells. This increases the proportion of antibodies that have the potential to bind the target antigen. Although immunization is thought of as a way to produce higher affinity antibodies (affinity maturation) in this case it is not. Rather, it can be considered as a way to shift the set of antigen combining sites towards the targets. This is also distinct from the concept of isotype switching where the functionality, as dictated by the constant portion of the heavy chain, is altered from the initial IgM isotype to another isotype such as IgG.

The third key feature that is crucial in the screening process is the use of multitarget screening. To a certain extent specificity is related to affinity. An example of this is the situation where an antigen has very limited tissue distribution and the affinity of the antibody is a key determinant of the specificity of the

antibody-the higher the affinity the more tissue specific the antibody and likewise an antibody with low affinity may bind to tissues other than the one of interest. Therefore, to address the specificity issue the antibodies are screened simultaneously against a variety of cells. In the examples below the hybridoma supernatants (representing the earliest stages of monoclonal antibody development), are tested against a number of cell lines to establish specificity as well as activity.

The antibodies are designed for therapeutic treatment of cancer in patients. Ideally the antibodies can be naked antibodies. They can also be conjugated to toxins. They can be used to target other molecules to the cancer. e.g. biotin conjugated enzymes. Radioactive compounds can also be used for conjugation.

The antibodies can be fragmented and rearranged molecularly. For example Fv fragments can be made; sFv-single chain Fv fragments; diabodies etc.

It is envisioned that these antibodies can be used for diagnosis, prognosis, and monitoring of cancer. For example the patients can have blood samples drawn for shed tumor antigens which can be detected by these antibodies in different formats such as ELISA assays, rapid test panel formats etc. The antibodies can be used to stain tumor biopsies for the purposes of diagnosis. In addition a panel of therapeutic antibodies can be used to test patient samples to determine if there are any suitable antibodies for therapeutic use.

Example one

In order to produce monoclonal antibodies specific for a tumor sample the method of selection of the appropriate hybridoma wells is complicated by the probability of selecting wells which will produce false positive signals. That is to say that there is the

likelihood of producing antibodies that can react against normal cells as well as cancer cells. To obviate this possibility one strategy is to mask the anti-normal antigen antibodies from the selection process. This can be accomplished by removing the anti-normal antibodies at the first stage of screening thereby revealing the presence of the desired antibodies. Subsequent limiting dilution cloning can delineate the clones that will not produce killing of control cells but will produce target cancer cell killing.

Biopsy specimens of breast, melanoma, and lung tumors were obtained and stored at -70°C until used. Single cell suspensions were prepared and fixed with -30°C, 70% ethanol, washed with PBS and reconstituted to an appropriate volume for injection. Balb/c mice were immunized with $2.5x10^5$-$1x10^6$ cells and boosted every third week until a final pre-fusion boost was performed three days prior to the splenectomy. The hybridomas were prepared by fusing the isolated splenocytes with Sp2/0 and NS1 myeloma partners. The supernatants from the fusions were tested for subcloning of the hybridomas.

Cells (including A2058 melanoma cells, CCD-12CoN fibroblasts, MCF-12A breast cells among others) were obtained from ATCC and cultured according to enclosed instructions. The HEY cell line was a gift from Dr. Inka Brockhausen. The non-cancer cells, e.g. CCD-12CoN fibroblasts and MCF-12A breast cells, were plated into 96-well microtitre plates (NUNC) 1 to 2 weeks prior to screening. The cancer cells, e.g. HEY, A2058, BT 483, and HS294t, were plated two or three days prior to screening.

The plated normal cells were fixed prior to use. The plates were washed with 100 microliters of PBS for 10 minutes at room temperature and then aspirated dry. 75 microliters of 0.01 percent glutaraldehyde diluted in PBS were added to each well for five minutes and then aspirated. The plates were washed with 100 microliters of

PBS three times at room temperature. The wells were emptied and 100 microliters of one percent human serum albumin in PBS was added to each well for one hour at room temperature. The plates were then stored at four degrees Celsius.

Prior to the transfer of the supernatant from the hybridoma plates the fixed normal cells were washed three times with 100 microliters of PBS at room temperature. After aspiration to the microliters of the primary hybridoma culture supernatants were transferred to the fixed cell plates and incubated for two hours at 37 degrees Celsius in a 8 percent CO_2 incubator. The hybridoma supernatants derived from melanoma was incubated with CCD-12 CoN cells and those derived from breast cancer were incubated with MCF-12a cells. After incubation the absorbed supernatant was divided into two 75 microliter portions and transferred to target cancer cell plates. Prior to the transfer the cancer cell plates were washed three times with 100 microliters of PBS. The supernatant from the CCD-12 CoN cells were transferred to the A2058 and the HS294t cells, whereas the supernatant from MCF-12A cells were transferred to the HEY and BT 483 cells. The cancer cells were incubated with the hybridoma supernatants for 18 hours at 37 degrees Celsisu in an 8 percent CO_2 incubator.

The Live/Dead cytotoxicity assay was obtained from Molecular Probes (Eu,OR). The assays were performed according to the manufacturer's instructions with the changes outlined below. The plates with the cells were washed once with 100 microliters of PBS at 37°C. 75 to 100 microliters of supernatant from the hybridoma microtitre plates were transferred to the cell plates and incubated in a 8% CO_2 incubator for 18-24 hours. Then, the wells that served as the all dead control were aspirated until empty and 50 microliters of 70% ethanol was added. The plate was then emptied by inverting and blotted dry. Room

temperature PBS was dispensed into each well from a multichannel squeeze bottle, tapped three times, emptied by inversion and then blotted dry. 50 microliters of the fluorescent Live/Dead dye diluted in PBS was added to each well and incubated at 37°C in a 5% CO_2 incubator for one hour. The plates were read in a Perkin-Elmer HTS7000 fluorescence plate reader and the data was analyzed in Microsoft Excel.

Four rounds of screening were conducted to produce single clone hybridoma cultures. For two rounds of screening the hybridoma supernatants were tested only against the cancer cells. In the last round of screening the supernatant was tested against a number of non-cancer cells as well as the target cells indicated in table 1. The antibodies were isotyped using a commercial isotyping kit.

A number of monoclonal antibodies were produced in accordance with the method of the present invention. These antibodies, whose characteristics are summarized in Table 1, are identified as 3BD-3, 3BD-6, 3BD-8, 3BD-9, 3BD-15, 3BD-25, 3BD-26 and 3BD-27. Each of the designated antibodies is produced by a hybridoma cell line deposited with the American Type Culture Collection at 10801 University Boulevard, Manassas, Va. having an ATCC Accession Number as follows:

Antibody	**ATCC Accession Number**
3BD-3	
3BD-6	
3BD-8	
3BD-9	
3BD-15	
3BD-25	
3BD-26	
3BD-27	

These antibodies are considered monoclonal after four rounds of limiting dilution cloning. The anti-melanoma antibodies did not produce significant cancer cell killing. The panel of anti-breast cancer antibodies killed 32-87% of the target cells and <1-3% of the control cells. The predominant isotype was IgG1 even though it was expected that the majority of anti-tumor antibodies would be directed against carbohydrate antigens, and thus, be of the IgM type. There is a high therapeutic index since most antibodies spare the control cells from cell death.

Table 1. Anti-Breast Cancer Antibodies

Clones	% Cell Death			
	Target for Anti-Breast Cancer Antibodies (HEY & A2058)	Normal Fibroblast Cells (CCD-12CoN)	Fibrocystic Breast Cells (MCF-12A)	Isotype
3BD-3	74.9%	3.7%	<1%	γ1, λ
3BD-6	68.5%	5.6%	<1%	γ1, λ
3BD-8	81.9%	4.5%	2.6%	γ1, κ
3BD-9	77.2%	7.9%	<1%	γ1, λ
3BD-15	87.1%	<1 %	<1%	γ1, λ
3BD-26	54.8%	3.3%	<1%	μ,κ
3BD-25	32.4%	3.6%	<1 %	γ1,κ
3BD-27	60.1%	8.3%	1.3%	γ1, κ

Example 2

In this example customized anti-cancer antibodies are produced by first obtaining samples of the patient's tumor. Usually this is from a biopsy specimen from a solid tumor or a blood sample from hematogenous tumors. The samples are prepared into single cell suspensions and fixed for injection into mice. After the completion of the immunization schedule the hybridomas are produced from the splenocytes. The hybridomas are screened against a variety

of cancer cell lines and normal cells in standard cytotoxicity assays. Those hybridomas that are reactive against cancer cell lines but are not reactive against normal non-transformed cells are selected for further propagation. Clones that were considered positive were ones that selectively killed the cancer cells but did not kill the non-transformed cells. The antibodies are characterized for a large number of biochemical parameters and then humanized for therapeutic use.

The melanoma tumor cells isolated and cell lines were cultured as described in Example 1. Balb/c mice were immunized according to the following schedule: 200,000 cells s.c. and i.p. on day 0, then 200,000 cells were injected i.p. on day 21, then 1,000,000 cells were injected on day 49, then 1,250,000 cells in Freund's Complete Adjuvant were injected i.p. on day 107, and then 200,000 cells were injected on day 120 i.p. and then the mice were sacrificed on day 123. The spleens were harvested and the splenocytes were divided into two aliquots for fusion with Sp2/0 (1LN) or NS-1 (2LN) myeloma partners using the methods outlined in example 1.

The screening was carried out 11 days after the fusion against A2058 melanoma cells and CCD-12CoN fibroblasts. Each pair of plates were washed with 100 microliters of room temperature PBS and then aspirated to near dryness. Then 50 microliters of hybridoma supernatant was added to the same wells on each of the two plates. The spent Sp2/0 supernatant was added to the control wells at the same volume and the plates were incubated for around 18 hours at 37 degrees Celsius at a 8%CO_2, 98% relative humidity incubator. Then each pair of plates were removed and in the positive control wells 50 microliters of 70% ethanol was substituted for the media for 4 seconds. The plates were then inverted and washed with room temperature PBS once and dried. Then 50uL of fluorescent live/dead dye diluted in PBS (Molecular Probes Live/Dead Kit) was added

for one hour and incubated at 37 degrees Celsius. The plates were then read in a Perkin-Elmer fluorescent plate reader and the data analyzed using Microsoft Excel. The wells that were considered positive were subcloned and the same screening process was repeated 13 days later and then 33 days later. The results of the last screening is outlined in Table 2 below. A number of monoclonal antibodies were produced in accordance with the method of the present invention. These antibodies, whose characteristics are summarized in Table 2, are identified as 1LN-1, 1LN-8, 1LN-12, 1LN-14, 2LN-21, 2LN-28, 2LN-29, 2LN-31, 2LN-33, 2LN-34 and 2LN-35. Each of the designated antibodies is produced by a hybridoma cell line deposited with the American Type Culture Collection at 10801 University Boulevard, Manassas, Va. having an ATCC Accession Number as follows:

Antibody	ATCC Accession Number
1LN-1	
1LN-8	
1LN-12	
1LN-14	
2LN-21	
2LN-28	
2LN-29	
2LN-31	
2LN-33	
2LN-34	
2LN-35	

Table 2, Anti-Melanoma Antibodies

Clones	% Cell Death	
	Target for Anti-Melanoma Antibodies (A2058)	Normal Fibroblast Cells (CCD-1 2CoN)
1LN-1	59.4%	<1 %
1LN-8	11.0%	5.0%
1LN-12	55.2%	1.4%
1LN-14	51.4%	<1%
2LN-21	72.0%	15.9%
2LN-28	66.6%	12.4%
2LN-29	78.2%	6.1%
2LN-31	100%	7.8%
2LN-33	94.2%	<1%
2LN-34	56.6%	11.2%
2LN-35	66.5%	6.6%

The table illustrates that clones from both the Sp2/0 and NS-1 fusions were able to produce antibodies that had a greater than 50% killing rate for cancerous cells and at the same time some of the clones were able to produce less than one percent killing of normal control fibroblasts.

Example 3

In this example antibodies were produced to several different breast tumor samples following the method of Example 2 in order to demonstrate the generality of producing customized antibodies. Biopsy specimens of breast tumors were obtained and stored at -70°C until used as noted in Example 1. Single cell suspensions were prepared for each specimen and fixed with -30°C, 70% ethanol, washed with PBS and reconstituted to an appropriate volume for injection. Female, 7-8 week old, A strain, H-2^d haplotype Balb/c mice (Charles River Canada,

St. Constant, QC, Can), were immunized with $2.5x10^5$-$1x10^6$ cells and boosted every third week until a final pre-fusion boost was performed three days prior to the splenectomy. The hybridomas were prepared by fusing the isolated splenocytes with Sp2/0 myeloma partners. The supernatants from the fusions were tested for subcloning of the hybridomas.

Hs574.T breast ductal carcinoma cells, A2058 melanoma cells, NCI-H460 human lung large cell carcinoma, NCI-H661 human lung large cell carcinoma, CCD-112CoN human colon fibroblasts, CCD-27sk human skin fibroblasts, MCF-12A human mammary epithelial cells, Hs574.mg human breast cells and other cell lines, were obtained from ATCC and cultured according to enclosed instructions. Both cancer and non-cancer cells were plated three to four days prior to screening.

The hybridomas were cultured for ten to twelve days after fusion and observed under the microscope. When 20 to 25% of the wells were over 80% confluent, the hybridoma supernatants were screened in a cytotoxicity assay. The hybridoma supernatants were divided into two 75 microliter portions; one portion was added to a target cancer cell plate and another to a non-cancer cell plate. Prior to transfer of hybridoma supernatants, the cell plates were washed three times with 100 microliters of PBS. The supernatant from the anti-breast cancer hybridomas were transferred to the Hs574.T and the Hs574.mg cells, whereas the supernatant from the anti-lung cancer hybridoma were transferred to the NCI-H460 and CCD-27SK cells. The

cancer cells were incubated with the hybridoma supernatants for 18 hours at 37 degrees Celsius in an 8 percent CO_2 incubator.

The Live/Dead cytotoxicity assay was obtained from Molecular Probes (Eugene,OR). The assays were performed according to the manufacturer's instructions with the changes outlined below. The plates with the cells were washed once with 100 microliters of PBS at 37°C. 75 to 100 microliters of supernatant from the hybridoma microtitre plates were transferred to the cell plates and incubated in a 8% CO_2 incubator for 18-24 hours. Then, the wells that served as the dead control cells were aspirated until empty and 50 microliters of 70% ethanol was added. The plate was then emptied by inverting and blotted dry. Room temperature PBS was dispensed into each well from a multichannel squeeze bottle, tapped three times, emptied by inversion and then blotted dry. 50 microliters of the fluorescent Live/Dead dye diluted in PBS was added to each well and incubated at 37°C in a 5% CO_2 incubator for one hour. The plates were read in a Perkin-Elmer HTS7000 fluorescence plate reader and the data was analyzed in Microsoft Excel (Microsoft, Redmond, WA).

Four rounds of screening were conducted to produce single clone hybridoma cultures. For two rounds of screening the hybridoma supernatants were tested only against the cancer cells. In the last round of screening the supernatant was tested against a number of non-cancer

cells as well as the target cells indicated in Table 3. The antibodies were isotyped using a commercial isotyping kit (Roche, Indianapolis, IN).

A number of monoclonal antibodies were produced in accordance with the method of the present invention. These antibodies, whose characteristics are summarized in Table 3, are identified as 4BD-1, 4BD-3, 4BD-6, 4BD-9, 4BD-13, 4BD-18, 4BD-20, 4BD-25, 4BD-37, 4BD-32, 4BD-26, 4BD-27, 4BD-28, 4BD-50, 6BD-1, 6BD-3, 6BD-5, 6BD-11, 6BD-25, 7BD-7, 7BD-12-1, 7BD-12-2, 7BD-13, 7BD-14, 7BD-19, 7BD-21, 7BD-24, 7BD-29, 7BD-30, 7BD-31, 7BDI-17, 7BDI-58, 7BDI-60 and 7BDI-62. Each of the designated antibodies is produced by a hybridoma cell line deposited with the American Type Culture Collection at 10801 University Boulevard, Manassas, Va. having an ATCC Accession Number as follows:

Antibody	ATCC Accession Number
4BD-1	
4BD-3	
4BD-6	
4BD-9	
4BD-13	
4BD-18	
4BD-20	
4BD-25	
4BD-37	
4BD-32	

4BD-26

4BD-27

4BD-28

4BD-50

6BD-1

6BD-3

6BD-5

6BD-11

6BD-25

7BD-7

7BD-12-1

7BD-12-2

7BD-13

7BD-14

7BD-19

7BD-21

7BD-24

7BD-29

7BD-30

7BD-31

7BDI-17

7BDI-58

7BDI-60

7BDI-62

These antibodies are considered monoclonal after four rounds of limiting dilution cloning. The panel of anti-breast cancer antibodies killed 15-79% of the target cells and <1-31% of the control cells. The majority of anti-

tumor antibodies were IgM type, suggesting they could be directed against carbohydrate antigens on the surface of tumor cells. There is a high therapeutic index since most of the antibodies do not cause the normal cells to undergo cell death.

These monoclonal antibodies are characterized for a number of immunological and biochemical parameters. A cell based enzyme linked immunosorbent assay (ELISA) was established for measuring the binding of the antibodies derived of each clones to different cell lines. Cells were seeded and grown on 96-well tissue culture plates. The plates were washed with 100 microliters of PBS. 100 microliters of cold 4 percent paraformaldehyde in PBS were added to each well for ten minutes and then aspirated. The plates were washed with PBS using a multichannel squeeze bottle . The wells were emptied and 100 microliters of blocking buffer (1 percent hydrocasein, 0.1 percent geletin in 50mM Tris-HCl buffer, pH 9.3) was added to each well for one hour at room temperature. The plates were washed three times with a buffer (0.05 percent Tween 20 in 10 mM PBS) at room temperature and then stored at -30 degrees Celsius with 100 microliters of the buffer. Prior to use the plates were thawed and the buffer was aspirated from each well. 75 microliters of hybridoma supernatant were added to each well and incubated for 60 minutes at room temperature. After the plates were washed with PBS using a multichannel squeeze bottle, 50 microliters of a

combination of peroxidase conjugated goat anti-mouse IgG and peroxidase conjugated donkey anti-mouse IgM (Jackson ImmunoResearch Lab, Inc., West Grove, PA.) was added and incubated for 30 minutes at room temperature. After the last wash, 50 microliters of orthophenylene diamine (OPD) (Sigma, St. Louis, MO) was added to each well and the optical density was read at 492 nm on the HTS7000 plate reader after adding equal volume of 1 N sulfuric acid. Different clones show different profiles in binding to different cells (Table 3). This indicates that they may target different cell surface antigen and further suggests the variable distribution of these antigen on the surface of cancer cells. Those binding only to cancer cells but not to normal cells could identify certain tumor-associated antigen.

Table 3. Anti-Breast Cancer Antibodies

Clones	Isotype	% Cell Death		Binding to cell lines				
		Hs574.T	Hs574.mg	Hs574.T	Hs574.mg	NCI-H460	CCD-27sk	A2058
6BD-1	μ, κ	38.2	5	0.8	0.5	0.6	0.3	ND*
6BD-3	μ, κ	79	12	0.35	0.25	0.24	0.14	ND
6BD-5	μ, κ	57.3	8	1.0	0.3	0.14	0.25	ND
6BD-11	μ, κ	52.3	11	0.15	0.1	0.17	0.1	ND
6BD-25	μ, κ	33.3	2	0.15	0.1	0.2	0.1	ND
4BD-26	μ, κ	27	1.8	0.5	ND	ND	<0.1	ND
4BD-27	μ, κ	19.6	<1	0.9	ND	ND	0.5	ND
4BD-28	μ, κ	26.4	<1	0.8	ND	ND	<0.1	ND
4BD-32	μ, κ	41.7	4	0.8	ND	ND	<0.1	ND
4BD-50	μ, κ	20	<1	0.8	ND	ND	<0.1	ND
4BD-1	μ, κ	23	31	0.6	ND	ND	<0.1	ND
4BD-3	μ, κ	29.7	8.2	1.7	ND	ND	0.1	ND
4BD-6	μ, κ	17	<1	0.9	ND	ND	<0.1	ND
4BD-9	μ, κ	15	<1	0.6	ND	ND	<0.1	ND
4BD-13	μ, κ	31	<1	1.2	ND	ND	<0.1	ND
4BD-18	μ, κ	23.3	2.4	0.7	ND	ND	0.12	ND
4BD-20	μ, κ	45	<1	0.95	ND	ND	<0.1	ND
4BD-25	μ, κ	26	14.16	1.8	ND	ND	0.1	ND
4BD-37	μ, κ	30	<1	0.8	ND	ND	<0.1	ND
7BD-7	μ, κ	24	3	0.8	0.3	1.4	0.26	ND
7BD-12-1	μ, κ	22	6	0.36	0.16	0.43	0.1	ND
7BD-12-2	μ, κ	31	2	0.2	0.2	0.2	0.2	0.2
7BD-13	μ, κ	29	12	0.1	0.15	0.2	0.1	0.2
7BD-14	μ, κ	32	13	0.4	0.4	0.6	0.3	0.5
7BD-19	μ, κ	20	4	1.3	0.4	0.43	0.2	ND
7BD-21	μ, κ	21	13	0.4	0.5	0.25	0.3	ND
7BD-24	μ, κ	32	15	0.3	0.1	0.14	0.15	ND
7BD-29	μ, κ	15	16	0.3	0.24	0.14	0.16	ND
7BD-30	μ, κ	23	13	0.34	0.24	0.2	0.16	ND
7BD-31	μ, κ	28	10	0.3	0.4	0.4	0.3	0.4

7BDI-17	μ, κ	23	<1	0.75	ND	ND	ND	ND
7BDI-58	γ1, κ	17.5	<1	0.77	ND	ND	ND	ND
7BDI-60	γ1, κ	15	<1	0.73	ND	ND	ND	ND
7BDI-62		15	5	0.55	ND	ND	ND	ND

*ND: not done.

Example 4

In this example customized anti-cancer antibodies are produced to a lung cancer sample by first obtaining samples of the patient's tumor preparing single cell suspensions which are then fixed for injection into mice as noted in Example 1. After the completion of the immunization schedule the hybridomas are produced from the splenocytes. The hybridomas are screened against a variety of cancer cell lines and normal cells in standard cytotoxicity assays. Those hybridomas that are reactive against cancer cell lines but are not reactive against normal non-transformed cells are selected for further propagation. Clones that were considered positive were ones that selectively killed the cancer cells but did not kill the non-transformed cells.

The lung cancer cells were isolated and cell lines were cultured as described in Example 1. Female, 7-8 week old, A strain, H-2^d haplotype Balb/c mice (Charles River Canada, St. Constant, QC, Can), were immunized with human lung cancer cells. The lung cancer cell suspensions were emulsified in an equal volume of Freund's complete adjuvant (FCA) for the first immunization and then in

Freund's incomplete adjuvant (FIA) for subsequent immunizations at 0, 21, 45 days. $5x10^5$ cells were used to immunize each mouse either through a subcutaneous or intra-peritoneal route. Immunized mice were sacrificed 3-4 days after the final immunization with human lung cancer cells at 148 days, given intra-peritoneally, in PBS at pH 7.4. The spleens were harvested and the splenocytes were divided into two aliquots for fusion with Sp2/0 myeloma partners using the methods outlined in Example 1.

The screening was carried out 10 days after the fusion against NCI-H460 and/or NCI-H661 cells and CCD-27SK fibroblasts. Each pair of plates were washed with 100 microliters of room temperature PBS and then aspirated to near dryness. Then 75 microliters of hybridoma supernatant was added per well on each of the two plates. The spent Sp2/0 supernatant was added to the control wells at the same volume and the plates were incubated for around 18 hours at 37 degrees Celsius at a 8%CO_2, 98% relative humidity incubator. Then each pair of plates was removed and in the positive control wells 50 microliters of 70% ethanol was substituted for the media for 4 seconds. The plates were then inverted and washed with room temperature PBS once and dried. Then 50 microliters of fluorescent live/dead dye diluted in PBS (Molecular Probes Live/Dead Kit) was added for one hour and incubated at 37 degrees Celsius. The plates were then read in a Perkin-Elmer fluorescent plate reader and the data analyzed using

Microsoft Excel. The wells that were considered positive were subcloned and the same screening process was repeated 6 days later and then 13 days later. The result of the last screening is outlined in Table 4 below. Antibodies were characterized for binding to different cell lines with a cellular ELISA according to the methods of Example 3. A number of monoclonal antibodies were produced in accordance with the method of the present invention. These antibodies, whose characteristics are summarized in Table 4, are identified as 5LAC2, 5LAC4, 5LAC20, and 5LAC23. Each of the designated antibodies is produced by a hybridoma cell line deposited with the American Type Culture Collection at 10801 University Boulevard, Manassas, Va. having an ATCC Accession Number as follows:

Antibody	**ATCC Accession Number**
5LAC2	
5LAC4	
5LAC20	
5LAC23.	

Table 4. Anti-Lung Cancer Antibodies

Clones	Isotype	% Cell Death					Binding to cell lines				
		Hs574.T	NCI-H460	NCI-H661	A2058	CCD-27sk	Hs574.T	Hs574.mg	NCI- H460	CCD-27sk	A2058
5LAC2	μ, K	30	7	45.3	23	<1	0.2	0.2	0.26	0.2	0.2
5LAC4	μ, K	21	11	20.5	23	3	0.7	0.9	1.7	0.8	0.9
5LAC20	μ, K	23	7	66.4	24	3	0.5	0.2	0.6	0.2	0.2
		23	8	57.6	25	5	0.6	0.6	0.6	0.6	0.6

*ND: not done

The table illustrates that clones were able to produce antibodies that had a greater than 7-67% killing rate for cancerous cells and at the same time some of the clones were able to produce less than five percent killing of normal control fibroblasts.

Example 5

In this example customized anti-cancer antibodies are produced to a patient's lung cancer cells, but cultured cells were used in the antibody development process to demonstrate the generality of the immunization process. The samples were prepared into single cell suspensions and fixed for injection into mice as noted in Example 1. After the completion of three rounds of immunization with cells derived directly from a patient's lung cancer, the mice were immunized twice with a human lung large cell carcinoma cell line (NCI-H460). Hybridomas were produced from splenocytes and the supernatants were screened against a variety of cancer cell lines and normal cells in standard cytotoxicity assays. Those hybridomas that were reactive against cancer cell lines but were not reactive against normal non-transformed cells were selected for further propagation. Clones that were considered positive were ones that selectively killed the cancer cells but did not kill the non-transformed cells. The antibodies are

characterized for a large number of biochemical parameters and then humanized for therapeutic use.

The lung tumor cells isolated and cell lines were cultured as described in Example 1. Balb/c mice, A strain with H-2^d haplotype from Charles River Canada, St. Constant, Quebec, Canada, female, 7-8 week old, were immunized with the human lung cancer cells emulsified in an equal volume of either Freund's complete adjuvant (FCA) for the first immunization and then in Freund's incomplete adjuvant (FIA) for subsequent immunizations at 0, 21, 45 days with $5x10^5$ cells. The mice were immunized with fixed NCI H460 cells, which were prepared from NCI H460 cells grown in T-75 cell culture flask by scraping mono-layer cells into cell suspensions at 105, 150 and 170 days. Immunized mice were sacrificed 3-4 days after the final immunization with NCI H460 cells, given intra-peritoneally, in phosphate buffered saline buffer (PBS), pH 7.4. The spleens were harvested and the splenocytes were divided into two aliquots for fusion with Sp2/0 myeloma partners using the methods outlined in Example 1.

The screening was carried out 10 days after the fusion against NCI H460 cells and CCD-27SK fibroblasts as described in Example 4. Antibodies were characterized for binding to different cell lines with a cellular ELISA according to the methods of Example 3.

The wells that were considered positive were subcloned and the same screening process was repeated 9 days and 18 days later. The results are outlined in Table 5 below. A number of monoclonal antibodies were produced in accordance with the method of the present invention. These antibodies, whose characteristics are summarized in Table 5, are identified as H460-1, H460-4, H460-5, H460-10, H460-14, H460-16-1, H460-16-2, H460-23 and H460-27. Each of the designated antibodies is produced by a hybridoma cell line deposited with the American Type Culture Collection at 10801 University Boulevard, Manassas, Va. having an ATCC Accession Number as follows:

Antibody	ATCC Accession Number
H460-1	
H460-4	
H460-5	
H460-10	
H460-14	
H460-16-1	
H460-16-2	
H460-23	
H460-27	

Table 5. Anti-Lung Cancer Antibodies

Clones	Isotype	% Cell Death				Binding to cell lines				
		NCI-H460	Hs574.	A2058	CCD-	Hs574.	Hs574.	NCI-	CCD-	A2058
H460-1	γ1,ê	16	30	23	<1	1.0	0.6	0.5	0.7	ND
H460-4		37	21	23	3	1.0	0.6	0.4	0.6	ND
H460-5	μ, κ	22.5	23	24	3	1.0	0.3	0.3	0.2	ND
H460-10	μ, κ	8	23	25	5	0.3	0.14	0.2	0.1	ND
H460-14	γ1,ê	17	ND	ND	4	1.1	0.6	0.4	0.54	ND
H460-16-1	γ1,ê	33	ND	ND	8	1.0	0.6	0.3	0.5	ND
H460-16-2	γ1,ê	22	ND	ND	3	1.0	0.6	0.3	0.7	ND
H460-22-1	γ1, ê	21	ND	ND	5	0.6	0.4	0.3	0.4	ND
H460-22-2	μ, κ	23	ND	ND	3	0.4	0.1	0.1	0.1	ND
H460-23	μ, κ	36	36	18	1	0.4	1.1	0.54	0.53	0.58
H460-27	μ, κ	33	31	16	8	0.3	0.4	0.4	0.3	0.4

*ND: not done

The table illustrates that clones were able to produce antibodies that had a greater than 15% killing rate for cancerous cells and at the same time some of the clones were able to produce less than eight percent killing of normal control fibroblasts.

The anti-cancer antibodies of the invention are useful for treating a patient with a cancerous disease when administered in admixture with a pharmaceutically acceptable adjuvant, for example normal saline, a lipid emulsion, albumen, phosphate buffered saline or the like and are administered in an amount effective to mediate treatment of said cancerous disease, for example with a

range of about 1 microgram per milliliter to about 1 gram per milliliter.

The method for treating a patient suffering from a cancerous disease may further include the use of conjugated anti-cancer antibodies and would this include conjugating patient specific anti-cancer antibodies with a member selected from the group consisting of toxins, enzymes, radioactive compounds, and hematogenous cells; and administering these conjugated antibodies to the patient; wherein said anti-cancer antibodies are administered in admixture with a pharmaceutically acceptable adjuvant, for example normal saline, a lipid emulsion, albumen, phosphate buffered saline or the like and are administered in an amount effective to mediate treatment of said cancerous disease, for example with a range of about 1 microgram per mil to about 1 gram per mil. In a particular embodiment, the anti-cancer antibodies useful in either of the above outlined methods may be a humanized antibody.

The anti-cancer antibodies of the invention are useful for treating a patient with a cancerous disease when administered in admixture with a pharmaceutically acceptable adjuvant, for example normal saline, a lipid emulsion, albumen, phosphate buffered saline or the like and are administered in an amount effective to mediate treatment of said cancerous disease, for example with a

range of about 1 microgram per mil to about 1 gram per mil.

The method for treating a patient suffering from a cancerous disease may further include the use of conjugated anti-cancer antibodies and would this include conjugating patient specific anti-cancer antibodies with a member selected from the group consisting of toxins, enzymes, radioactive compounds, and hematogenous cells; and

administering these conjugated antibodies to the patient; wherein said anti-cancer antibodies are administered in admixture with a pharmaceutically acceptable adjuvant, for example normal saline, a lipid emulsion, albumen, phosphate buffered saline or thé like and are administered in an amount effective to mediate treatment of said cancerous disease, for example with a range of about 1 microgram per mil to about 1 gram per mil. In a particular embodiment, the anti-cancer antibodies useful in either of the above outlined methods may be a humanized antibody.

CLAIMS

What is claimed is:

Claim 1. A method for treating a patient suffering from a cancerous disease comprising:

administering to said patient anti-cancer antibodies or fragments thereof produced in accordance with a method for the production of individually customized anti-cancer antibodies which are useful in treating a cancerous disease, said antibodies including a subset of antibodies or fragments thereof characterized as being cytotoxic against cells of a cancerous tissue, said subset being essentially benign to non-cancerous cells;

wherein one or lord a.dibodies or fragments therdmf selected from said subset are placed in admixture with a pharmaceutically acceptable adjuvant and are administered in an amount effective to mediate treatment of said cancerous disease;

said one or more antibodies or fragments thereof being selected from the group consisting of a 1LN-8, 4BD-1, a 4BD-3, a 4BD-6, a 4BD-9, a 4BD-13, a 4BD-18, a 4BD-20, a 4BD-25, a 4BD-26, a 4BD-27, a 4BD-28, a 4BD-32, a 4BD-37, a 4BD-50, a 6BD-1, a 6BD-3, a 6BD-5, a 6BD-11, a 6BD-25, a 7BD-7, a 7BD-12-1, a 7BD-12-2, a 7BD-13, a 7BD-14, a 7BD-19, a 7BD-21, a 7BD-24, a 7BD-29, a 7BD-30, a 7BD-31, a 7BDI-17, a 7BDI-58, a 7BDI-60, a 7BDI-62, a 5LAC2, a 5LAC4, a 5LAC20, a 5LAC23, a H460-1, a H460-4, a

H460-5, a H460-10, a H460-14, a H460-16-1, a H460-16-2, a H460-23 and a H460-27 monoclonal antibody or combinations thereof.

Claim 2. The method for treating a patient suffering from a cancerous disease in accordance with claim 1, wherein said one or more antibodies or fragments thereof selected from said subset are humanized.

Claim 3. The method for treating a patient suffering from a cancerous disease in accordance with claim 1 comprising:

conjugating said subset of antibodies or fragments thereof with a member selected from the group consisting of toxins, enzymes, radioactive compounds, and hematogenous cells; and

administering conjugated antibodies or fragments thereof to said patient;

wherein said conjugated antibodies are placed in admixture with a pharmaceutically acceptable adjuvant and are administered in an amount effective to mediate treatment of said cancerous disease.

Claim 4. The method of claim 3, wherein said one or more antibodies or fragments thereof selected from said subset are humanized.

Claim 5. The method for treating a patient suffering from a cancerous disease in accordance with claim 1 wherein:

the cytotoxicity of said antibodies or fragments thereof is mediated through antibody dependent cellular toxicity.

Claim 6. The method for treating a patient suffering from a cancerous disease in accordance with claim 1 wherein:

the cytotoxicity of said antibodies or fragments thereof is mediated through complement dependent cellular toxicity.

Claim 7. The method for treating a patient suffering from a cancerous disease in accordance with claim 1 wherein:

the cytotoxicity of said antibodies or fragments thereof is mediated through catalyzing of the hydrolysis of cellular chemical bonds.

Claim 8. The method for treating a patient suffering from a cancerous disease in accordance with claim 1 wherein:

the cytotoxicity of said antibodies or fragments thereof is mediated through producing an immune response against putative cancer antigens residing on tumor cells.

Claim 9. The method for treating a patient suffering from a cancerous disease in accordance with claim 1 wherein:

the cytotoxicity of said antibodies or fragments thereof is mediated through targeting of cell membrane proteins to interfere with their function.

Claim 10. The method for treating a patient suffering from a cancerous disease in accordance with claim 1 wherein:

the cytotoxicity of said antibodies or fragments thereof is mediated through production of a conformational change in a cellular protein effective to produce a signal to initiate cell-killing.

Claim 11. The method for treating a patient suffering from a cancerous disease in accordance with claim 1 wherein:

said method of production utilizes a tissue sample containing cancerous and non-cancerous cells obtained from a particular individual.

Claim 12. A method for treating a patient suffering from a cancerous disease comprising:

administering to said patient anti-cancer antibodies or fragments thereof produced in accordance with a method for the production of individually customized anti-cancer antibodies which are useful in treating a cancerous

disease, said antibodies including a subset of antibodies or fragments thereof characterized as being cytotoxic against cells of a cancerous tissue, said subset being essentially benign to non-cancerous cells;

wherein one or more antibodies or fragments thereof selected from said subset are placed in admixture with a pharmaceutically acceptable adjuvant and are administered in an amount effective to mediate treatment of said cancerous disease;

said one or more antibodies or fragments thereof produced by a hybridoma cell line having an ATCC Accession Number selected from the group consisting of (to be provided before publication) or combinations thereof.

Claim 13. The method for treating a patient suffering from a cancerous disease in accordance with claim 12, wherein said one or more antibodies or fragments thereof selected from said subset are humanized.

Claim 14. The method for treating a patient suffering from a cancerous disease in accordance with claim 12 comprising:

conjugating said subset of antibodies or fragments thereof with a member selected from the group consisting of toxins, enzymes, radioactive compounds, and hematogenous cells; and

administering conjugated antibodies or fragments thereof to said patient;

wherein said conjugated antibodies are placed in admixture with a pharmaceutically acceptable adjuvant and are administered in an amount effective to mediate treatment of said cancerous disease.

Claim 15. The method of claim 14, wherein said one or more antibodies or fragments thereof selected from said subset are humanized.

Claim 16. The method for treating a patient suffering from a cancerous disease in accordance with claim 12 wherein:

the cytotoxicity of said antibodies or fragments thereof is mediated through antibody dependent cellular toxicity.

Claim 17. The method for treating a patient suffering from a cancerous disease in accordance with claim 12 wherein:

the cytotoxicity of said antibodies or fragments thereof is mediated through complement dependent cellular toxicity.

Claim 18. The method for treating a patient suffering from a cancerous disease in accordance with claim 12 wherein:

the cytotoxicity of said antibodies or fragments thereof is mediated through catalyzing of the hydrolysis of cellular chemical bonds.

Claim 19. The method for treating a patient suffering from a cancerous disease in accordance with claim 12 wherein:

the cytotoxicity of said antibodies or fragments thereof is mediated through producing an immune response against putative cancer antigens residing on tumor cells.

Claim 20. The method for treating a patient suffering from a cancerous disease in accordance with claim 12 wherein:

the cytotoxicity of said antibodies or fragments thereof is mediated through targeting of cell membrane proteins to interfere with their function.

Claim 21. The method for treating a patient suffering from a cancerous disease in accordance with claim 12 wherein:

the cytotoxicity of said antibodies or fragments thereof is mediated through production of a conformational change in a cellular protein effective to produce a signal to initiate cell-killing.

Claim 22. The method for treating a patient suffering from a cancerous disease in accordance with claim 12 wherein:

said method of production utilizes a tissue sample containing cancerous and non-cancerous cells obtained from a particular individual.

Claim 23. Anti-cancer antibodies or fragments thereof selected from the group consisting of a 1LN-8, 4BD-1, a 4BD-3, a 4BD-6, a 4BD-9, a 4BD-13, a 4BD-18, a 4BD-20, a 4BD-25, a 4BD-26, a 4BD-27, a 4BD-28, a 4BD-32, a 4BD-37, a 4BD-50, a 6BD-1, a 6BD-3, a 6BD-5, a 6BD-11, a 6BD-25, a 7BD-7, a 7BD-12-1, a 7BD-12-2, a 7BD-13, a 7BD-14, a 7BD-19, a 7BD-21, a 7BD-24, a 7BD-29, a 7BD-30, a 7BD-31, a 7BDI-17, a 7BDI-58, a 7BDI-60, a 7BDI-62, a 5LAC2, a 5LAC4, a 5LAC20, a 5LAC23, a H460-1, a H460-4, a H460-5, a H460-10, a H460-14, a H460-16-1, a H460-16-2, a H460-23 and a H460-27 monoclonal antibody or combinations thereof.

Claim 24. Anti-cancer antibodies or fragments thereof produced by a hybridoma cell line having an ATCC Accession Number selected from the group consisting of (to be provided before publication).

Claim 25. The use of a composition for treating a patient suffering from a cancerous disease by administration of an effective amount of said composition to a patient to mediate treatment of said cancerous disease, wherein said composition comprises one or more antibodies or fragments thereof selected from a subset of said antibodies or fragments in admixture with a pharmaceutically acceptable adjuvant, said anti-cancer antibodies or fragments thereof produced in accordance with a method for the production of individually customized anti-cancer antibodies which are useful in treating cancerous disease, said subset of antibodies or fragments thereof characterized as being cytotoxic against cells of a cancerous tissue, as being essentially benign to non-cancerous cells and being selected from the group consisting of a 1LN-8, 4BD-1, a 4BD-3, a 4BD-6, a 4BD-9, a 4BD-13, a 4BD-18, a 4BD-20, a 4BD-25, a 4BD-26, a 4BD-27, a 4BD-28, a 4BD-32, a 4BD-37, a 4BD-50, a 6BD-1, a 6BD-3, a 6BD-5, a 6BD-11, a 6BD-25, a 7BD-7, a 7BD-12-1, a 7BD-12-2, a 7BD-13, a 7BD-14, a 7BD-19, a 7BD-21, a 7BD-24, a 7BD-29, a 7BD-30, a 7BD-31, a 7BDI-17, a 7BDI-58, a 7BDI-60, a 7BDI-62, a 5LAC2, a 5LAC4, a 5LAC20, a 5LAC23, a H460-1, a H460-4, a H460-5, a H460-10, a H460-14, a H460-16-1, a H460-16-2, a H460-23 and a H460-27 monoclonal antibody or combinations thereof.

Claim 26. The use of a composition for treating a patient suffering from a cancerous disease by administration of an effective amount of the composition to the patient to mediate treatment of said cancerous disease, wherein said composition comprises one or more antibodies or fragments thereof from a subset of antibodies or fragments thereof characterized as being cytotoxic against cells of a cancerous tissue and essentially benign to non-cancerous cells placed in admixture with a pharmaceutically acceptable adjuvant, said one or more antibodies or fragments thereof produced by a hybridoma cell line having an ATCC Accession Number selected from the group consisting of (to be provided before publication) or combinations thereof.

Received by the International Bureau on 28 April 2003 (28.04.2003)
original claims 1-26 replaced by amended claims 1-25 (04 pages).

What is claimed is:

Claim 1. A method for treating a patient suffering from a cancerous disease comprising:

administering to said patient anti-cancer antibodies or fragments thereof produced in accordance with a method for the production of individually customized anti-cancer antibodies which are useful in treating a cancerous disease, said antibodies including a subset of antibodies or fragments thereof characterized as being cytotoxic against cells of a cancerous tissue, said subset being essentially benign to non-cancerous cells;

wherein one or more antibodies or fragments thereof selected from said subset are placed in admixture with a pharmaceutically acceptable adjuvant and are administered in an amount effective to mediate treatment of said cancerous disease;

said one or more antibodies or fragments thereof being selected from the group consisting of a 1LN-8, 4BD-1, a 4BD-3, a 4BD-6, a 4BD-9, a 4BD-13, a 4BD-18, a 4BD-20, a 4BD-25, a 4BD-26, a 4BD-27, a 4BD-28, a 4BD-32, a 4BD-37, a 4BD-50, a 6BD-1, a 6BD-3, a 6BD-5, a 6BD-11, a 6BD-25, a 7BD-7, a 7BD-12-1, a 7BD-12-2, a 7BD-13, a 7BD-14, a 7BD-19, a 7BD-21, a 7BD-24, a 7BD-29, a 7BD-30, a 7BD-31, a 7BDI-17, a 7BDI-58, a 7BDI-60, a 7BDI-62, a 5LAC2, a 5LAC4, a 5LAC20, a 5LAC23, a H460-1, a H460-4, a

H460-5, a H460-10, a H460-14, a H460-16-1, a H460-16-2, a H460-22-1, a H460-23 and a H460-27 monoclonal antibody or combinations thereof.

Claim 2. The method for treating a patient suffering from a cancerous disease in accordance with claim 1, wherein said one or more antibodies or fragments thereof selected from said subset are humanized.

Claim 3. The method for treating a patient suffering from a cancerous disease in accordance with claim 1 comprising:

conjugating said subset of antibodies or fragments thereof with a member selected from the group consisting of toxins, enzymes, radioactive compounds, and hematogenous cells; and

administering conjugated antibodies or fragments thereof to said patient;

wherein said conjugated antibodies are placed in admixture with a pharmaceutically acceptable adjuvant and are administered in an amount effective to mediate treatment of said cancerous disease.

Claim 4. The method of claim 3, wherein said one or more antibodies or fragments thereof selected from said subset are humanized.

Claim 22. The method for treating a patient suffering from a cancerous disease in accordance with claim 12 wherein:

said method of production utilizes a tissue sample containing cancerous and non-cancerous cells obtained from a particular individual.

Claim 23. Anti-cancer antibodies or fragments thereof selected from the group consisting of a 1LN-8, 4BD-1, a 4BD-3, a 4BD-6, a 4BD-9, a 4BD-13, a 4BD-18, a 4BD-20, a 4BD-25, a 4BD-26, a 4BD-27, a 4BD-28, a 4BD-32, a 4BD-37, a 4BD-50, a 6BD-1, a 6BD-3, a 6BD-5, a 6BD-11, a 6BD-25, a 7BD-7, a 7BD-12-1, a 7BD-12-2, a 7BD-13, a 7BD-14, a 7BD-19, a 7BD-21, a 7BD-24, a 7BD-29, a 7BD-30, a 7BD-31, a 7BDI-17, a 7BDI-58, a 7BDI-60, a 7BDI-62, a 5LAC2, a 5LAC4, a 5LAC20, a 5LAC23, a H460-1, a H460-4, a H460-5, a H460-10, a H460-14, a H460-16-1, a H460-16-2, a H460-22-1, a H460-23 and a H460-27 monoclonal antibody or combinations thereof.

Claim 24. Anti-cancer antibodies or fragments thereof produced by a hybridoma cell line having an ATCC Accession Number selected from the group
consisting of (to be provided before publication).

Claim 25. The use of a composition for treating a patient suffering from a cancerous disease by administration of an effective amount of said composition to a patient to mediate treatment of said cancerous disease, wherein said composition comprises one or more antibodies or fragments thereof selected from a subset of said antibodies or fragments in admixture with a pharmaceutically acceptable adjuvant, said anti-cancer antibodies or fragments thereof produced in accordance with a method for the production of individually customized anti-cancer antibodies which are useful in treating cancerous disease, said subset of antibodies or fragments thereof characterized as being cytotoxic against cells of a cancerous tissue, as being essentially benign to non-cancerous cells and being selected from the group consisting of a 1LN-8, 4BD-1, a 4BD-3, a 4BD-6, a 4BD-9, a 4BD-13, a 4BD-18, a 4BD-20, a 4BD-25, a 4BD-26, a 4BD-27, a 4BD-28, a 4BD-32, a 4BD-37, a 4BD-50, a 6BD-1, a 6BD-3, a 6BD-5, a 6BD-11, a 6BD-25, a 7BD-7, a 7BD-12-1, a 7BD-12-2, a 7BD-13, a 7BD-14, a 7BD-19, a 7BD-21, a 7BD-24, a 7BD-29, a 7BD-30, a 7BD-31, a 7BDI-17, a 7BDI-58, a 7BDI-60, a 7BDI-62, a 5LAC2, a 5LAC4, a 5LAC20, a 5LAC23, a H460-1, a H460-4, a H460-5, a H460-10, a H460-14, a H460-16-1, a H460-16-2, a H460-22-1, a H460-23 and a H460-27 monoclonal antibody or combinations thereof.

STATEMENT UNDER ARTICLE 19 (1)

The amendments include:

(1) amendments to claim 1 to correct editorial errors.

(2) amendments to claims 1, 23 and 25 to insert reference to "a H460-22-1," in the three claims, support for this appearing in Table 5 on page 37, line 13.

To provide consistency with the above, a new disclosure page 36 is included with the amended claim pages.

Finally, attached hereto are copies of submissions and acknowledgement of the submission of Deposited Material and certain ATCC Numbers in relation to a related and counterpart U.S. application number 09/727,361 as provided to us by the instructing U.S. Attorney. This material also makes reference to H460-22-1.

The cell line referred to in the ATCC material is found in the disclosure at the noted pages hereinbelow:

Cell Line	Reference Pages
1LN-8	22 (23)
5LAC20	33 (33)
3BD-26	19 (20)
3BD-8	19 (20)
7BD-14	27 (30)
3BD-27	19 (20)
H460-27	36 (37)
H460-23	36 (37)
H460-16-2	36 (37)
H460-22-1	36 (37) (amended as above)
7BDI-60	27 (31)

The first reference page number being reference to the page where the cell line is listed, whereas the second bracketed number is the page of the table in which the cell line appears.

Appropriate amendments to the disclosure to add the ATCC numbers will be effected in due course.

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

IN RE APPLICANT	: Young et al.
INVENTION	: **Individualized Anti-Cancer Antibodies**
SERIAL NUMBER	: 09/727,361
FILING DATE	: November 29, 2000
EXAMINER	: Susan Ungar
GROUP ART UNIT	: 1642
OUR FILE NO.	: 2056.009

To: The Commissioner of Patents and Trademarks
Washington, D.C. 20231

CORROBORATION FOR DEPOSITED MATERIALS

I, Ferris H. Lander, a person in a position to corroborate the identity of the hybridoma cell lines 1LN-8 (shown in the table on page 30), 3BD-8 (shown in the table on page 26), 3BD-26 (shown in the table on page 26), 3BD-27 (shown in the table on page 26), H460-27 (shown in the table on page 45), H460-23 (shown in the table on page 45), 7BD-14 (shown in the table on page 35) and 5LAC20 (shown in the table on page 41) which were deposited, in accordance with the Budapest Treaty, with the American Type Culture Collection (ATCC), 10801 University Blvd., Manassas, VA 20110-2209 on November 21, 2000 under Accession Numbers PTA-2693, PTA-2696, PTA-2695, PTA-2698, PTA-2699, PTA-2700, PTA-2697 and PTA 2694 respectively. And additionally, the hybridoma cell lines H460-16-2 (shown in the table on page 45) and 7BDI-60 (shown in the table on page 39) which were deposited on September 4, 2002 under Accession Numbers PTA-4621 and PTA-4623 respectively, do hereby state that the deposited hybridoma are the same hybridoma cell lines disclosed and claimed in the above-referenced patent application.

Respectfully submitted,

Ferris H. Lander
Registration # 43,377

McHale & Slavin, P.A.
4440 PGA Blvd., Suite 402
Palm Beach Gardens, FL 33410
(561) 625-6575 (Voice)
(561) 625-6572 (Fax)

ATCC

10801 University Blvd • Manassas, VA 20110-2209 • Telephone: 703-365-2700 • FAX: 703-365-2745

BUDAPEST TREATY ON THE INTERNATIONAL RECOGNITION OF
THE DEPOSIT OF MICROORGANISMS FOR THE PURPOSES OF PATENT PROCEDURE

INTERNATIONAL FORM

RECEIPT IN THE CASE OF AN ORIGINAL DEPOSIT ISSUED PURSUANT TO RULE 7.3
AND VIABILITY STATEMENT ISSUED PURSUANT TO RULE 10.2

To: (Name and Address of Depositor or Attorney)

Arius Research Inc.
Attn: Lisa Cechetto
6299 Airport Road
Mississauga, ON
L4V 1N3
Canada

Deposited on Behalf of: Arius Research Inc.

Identification Reference by Depositor:	**Patent Deposit Designation**
Mouse hybridoma cell line: 1LN8	PTA-2693
Mouse hybridoma cell line: 5LAC20	PTA-2694
Mouse hybridoma cell line: 3BD26	PTA-2695
Mouse hybridoma cell line: 3BD8	PTA-2696
Mouse hybridoma cell line: 7BD14	PTA-2697
Mouse hybridoma cell line: 3BD27	PTA-2698
Mouse hybridoma cell line: H460-27	PTA-2699
Mouse hybridoma cell line: H460-23	PTA-2700

The deposits were accompanied by: __ a scientific description _ a proposed taxonomic description indicated above. The deposits were received November 21, 2000 by this International Depository Authority and have been accepted.

AT YOUR REQUEST: X We will inform you of requests for the strains for 30 years.

The strains will be made available if a patent office signatory to the Budapest Treaty certifies one's right to receive, or if a U.S. Patent is issued citing the strains, and ATCC is instructed by the United States Patent & Trademark Office or the depositor to release said strains.

If the cultures should die or be destroyed during the effective term of the deposit, it shall be your responsibility to replace them with living cultures of the same.

The strains will be maintained for a period of at least 30 years from date of deposit, or five years after the most recent request for a sample, whichever is longer. The United States and many other countries are signatory to the Budapest Treaty.

The viability of the cultures cited above was tested November 30, 2000. On that date, the cultures were viable.

International Depository Authority: American Type Culture Collection, Manassas, VA 20110-2209 USA.

Signature of person having authority to represent ATCC:

Frank Simione, Director, Patent Depository

Date: December 21, 2000

cc: Mr. Ferris Lander (Ref: Docket or Case No.: 09/415,278)

ATCC

10801 University Blvd • Manassas, VA 20110-2209 • Telephone: 703-365-2700 • FAX: 703-365-2745

BUDAPEST TREATY ON THE INTERNATIONAL RECOGNITION OF THE DEPOSIT OF MICROORGANISMS FOR THE PURPOSES OF PATENT PROCEDURE

INTERNATIONAL FORM

RECEIPT IN THE CASE OF AN ORIGINAL DEPOSIT ISSUED PURSUANT TO RULE 7.3 AND VIABILITY STATEMENT ISSUED PURSUANT TO RULE 10.

To: (Name and Address of Depositor or Attorney)

Arius Research Inc.
Attn: Jean de Sousa-Hitzler
55 York Street 16th Floor
Toronto, Ontario
Canada M5J 1R7

Deposited on Behalf of: Arius Research Inc.

Identification Reference by Depositor:	Patent Deposit Designation
Mouse Hybridoma: H460-16-2	PTA-4621
Mouse Hybridoma: H460-22-1	PTA-4622
Mouse Hybridoma: 7BD1-60	PTA-4623

The deposits were accompanied by: __ a scientific description _ a proposed taxonomic description indicated above. The deposits were received September 4, 2002 by this International Depository Authority and have been accepted.

AT YOUR REQUEST: X We will inform you of requests for the strains for 30 years.

The strains will be made available if a patent office signatory to the Budapest Treaty certifies one's right to receive, or if a U.S. Patent is issued citing the strains, and ATCC is instructed by the United States Patent & Trademark Office or the depositor to release said strains.

If the cultures should die or be destroyed during the effective term of the deposit, it shall be your responsibility to replace them with living cultures of the same.

The strains will be maintained for a period of at least 30 years from date of deposit, or five years after the most recent request for a sample, whichever is longer. The United States and many other countries are signatory to the Budapest Treaty.

The viability of the cultures cited above was tested September 6, 2002. On that date, the cultures were viable.

International Depository Authority: American Type Culture Collection, Manassas, VA 20110-2209 USA.

Signature of person having authority to represent ATCC:

Marie Harris

Marie Harris, Patent Specialist, ATCC Patent Depository

Date: October 9, 2002

cc: Mr. Ferris Lander
(Ref: Docket or Case No.: 2056.009 & US Serial No. 09/727361)

INTERNATIONAL SEARCH REPORT

tional Application No
PCT/CA 01/01838

A. CLASSIFICATION OF SUBJECT MATTER
IPC 7 A61K39/395 A61K47/48 A61P35/00

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 7 G01N C07K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)
MEDLINE, BIOSIS, EPO-Internal, WPI Data, PAJ

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	US 2001/009665 A1 (YOUNG DAVID S F ET AL) 26 July 2001 (2001-07-26) the whole document	1-26
X	US 2001/003777 A1 (YOUNG DAVID S F ET AL) 14 June 2001 (2001-06-14) the whole document	1-26
X	US 6 180 357 B1 (YOUNG DAVID S F ET AL) 30 January 2001 (2001-01-30) the whole document	1-26

-/--

[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

° Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
10 January 2003	16/01/2003

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	COVONE-VAN HEES, M

INTERNATIONAL SEARCH REPORT

Ional Application No
PCT/CA 01/01838

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication,where appropriate, of the relevant passages	Relevant to claim No.
X	WO 95 20401 A (UNIV BOSTON) 3 August 1995 (1995-08-03) page 17, line 16-27 page 29, line 4-20 example 13 claims 30,31,38-40	1-6, 11-17, 22-26

INTERNATIONAL SEARCH REPORT

ıational application No.
PCT/CA 01/01838

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. [X] Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

Although claims 1-22, 25,26 are directed to a method of treatment of the human/animal body, the search has been carried out and based on the alleged effects of the compound/composition.

2. [ ] Claims Nos.:
because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:

3. [ ] Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. [ ] As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.

2. [ ] As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. [ ] As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:

4. [ ] No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

[ ] The additional search fees were accompanied by the applicant's protest.

[ ] No protest accompanied the payment of additional search fees.

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
US 2001009665 A1	26-07-2001	US 6180357 B1	30-01-2001
		US 2002041877 A1	11-04-2002
		US 2001003777 A1	14-06-2001
US 2001003777 A1	14-06-2001	US 6180357 B1	30-01-2001
		US 2002041877 A1	11-04-2002
		US 2001009665 A1	26-07-2001
US 6180357 B1	30-01-2001	US 2002041877 A1	11-04-2002
		US 2001009665 A1	26-07-2001
		US 2001003777 A1	14-06-2001
WO 9520401 A	03-08-1995	AU 1736495 A	15-08-1995
		EP 1231268 A2	14-08-2002
		EP 0744958 A1	04-12-1996
		US 6335163 B1	01-01-2002
		WO 9520401 A1	03-08-1995
		US 5789208 A	04-08-1998
		US 2001049107 A1	06-12-2001